PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/16, 38/17, 9/00

A1

(11) International Publication Number: **WO 99/43340**

(43) International Publication Date: 2 September 1999 (02.09.99)

(21) International Application Number: PCT/EP99/01068

(22) International Filing Date: 18 February 1999 (18.02.99)

(30) Priority Data:
MI98A000356 24 February 1998 (24.02.98) IT

(71) Applicant *(for all designated States except US)*: ZETESIS S.P.A. [IT/IT]; Galleria del Corso, 2, I–20122 Milano (IT).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BARTORELLI, Alberto [IT/IT]; Via G. D'Arezzo, 6, I–20145 Milano (IT). SANTI, Cesare [IT/IT]; Galleria del Corso, 2, I–20122 Milano (IT).

(74) Agent: MINOJA, Fabrizio; Bianchetti Bracco Minoja S.r.l., Via Rossini, 8, I–20122 Milano (IT).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ORAL COMPOSITIONS AT LOW DOSAGE OF CYTOTOXIC PROTEINS

(57) Abstract

Pharmaceutical compositions for the oral and sublingual administration containing proteins extractable from mammalian liver. Such proteins include the proteins marked with UK101 and UK114 and described in WO 92/10197 and WO 96/02567 as well as ubiquitin, contained in the UK101 extract.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

ORAL COMPOSITIONS AT LOW DOSAGE OF CYTOTOXIC PROTEINS

The present invention relates to pharmaceutical compositions for oral and sublingual administration containing proteins extractable from mammalian liver.

Such proteins include the proteins referred to as UK101 and UK114 and described in WO 92/10197 and WO 96/02567 as well as ubiquitin, contained in the UK101 extract.

It has been observed that the subcutaneous administration of UK101 and UK114 induces a clear cytotoxicity in serum of both healthy subjects and in tumor carriers subjects.

The main responsible for this effect is the 14Kd protein (UK114) contained in the protein extract UK101. The UK114 amino acidic sequence has been described in FEBS Let. 393, 147-150, 1996.

At present, clinical experimentations to verify the UK101 and UK114 therapeutic efficacy are in progress. To this order, patients suffering from colon and breast carcinoma are treated with UK101 and UK114 subcutaneous injections, at dosages ranging from 1 to 10 mg/week.

The protein nature of the active principle obviously forces the parenteral route

In fact, at present it is not known the possibility to administer proteins orally, due to their high metabolic instability.

To avert this inconvenience, it has been suggested several answers such as the use of suitable carriers or the encapsulation in liposomes, but until now the results have been unfavourable.

Now it has been found that it is possible to administer UK114 and UK101 or ubiquitin orally at low dosages, preferably for sublingual

administration, thus inducting a seric cytotoxicity comparable to or higher than that obtainable for subcutaneous administration.

The oral/sublingual route also shows clear advantages in practicality and safety terms.

The invention therefore provides pharmaceutical compositions for UK101 and UK114 oral and/or sublingual administration.

Suitable administration forms include, for example, aqueous suspensions to administer in drops, granules or sublingual tablets by a quickly disintegration, effervescent or chewable tablets or its equivalent forms.

The compositions of the invention can be prepared using conventional techniques and excipients, widely known in the pharmaceutical field.

The UK101 and UK114 unitary dosages can range from 10×10^{-4} to 10×10^{-15}g.

In the case of solutions to administrate in drops, the concentration of the active principle can range from 10^{-5} to 10^{-10} M. The administration of 10-15 drops a day proved to be sufficient to induce cytotoxicity in the patient serum, which can be evidenced on carcinoma cells Jurkat and Kato III according to standard protocols.

EXAMPLE

17 patients suffering from tumors in advanced phase (8 sarcoma, 4 breast carcinoma, 2 pancreas carcinoma and 3 colon carcinoma) have been treated with 5-20 drops/day of an 1% hydroalcoholic solution of ethanol in a UK101 concentration of 10^{-6} M.

The treatment continued for 30 consecutive days, involved in 70% of the cases an improvement in the subjective conditions of the patients, particularly a decrease in the painful symptomatology, a decrease in the tumor mass in 20% of the cases, joined to a cytotoxicity visible in the

patients serum on cell lines Jurkat and Kato III.

CLAIMS

1 Oral pharmaceutical compositions containing as active principle a protein selected from ubiquitin, UK114 and UK101.

2 Composition according to claim 1, suitable for the sublingual administration.

3 Compositions according to claim 2, in the form of drops , granules or sublingual tablets.

4 Compositions according to any one of the previous claims, containing from 10^{-4} to 10^{-15} g of UK101, UK114 and ubiquitin for unitary.

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/EP 99/01068

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/16 A61K38/17 A61K9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 92 10197 A (A. BARTORELLI ET AL.) 25 June 1992 (1992-06-25) cited in the application page 4, line 1 - line 12; claims ---	1-4
A	WO 96 02567 A (ZETESIS S.P.A.) 1 February 1996 (1996-02-01) cited in the application page 3, line 10 - line 16; claims; example 1 ---	1-4
A	GB 989 826 A (LABORATOIRE FRANCAIS DE SPECIALITES PHYSIOLOGIQUES ET HYGIENIQUES) 22 April 1965 (1965-04-22) claims 1,7 -----	1-4

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 August 1999	18/08/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ryckebosch, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ional Application No
PCT/EP 99/01068

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9210197 A	25-06-1992	IT	1244879 B	12-09-1994
		AT	122890 T	15-06-1995
		AU	661287 B	20-07-1995
		AU	9035791 A	08-07-1992
		CA	2098113 A	11-06-1992
		CZ	9301116 A	13-04-1994
		DE	69110060 D	29-06-1995
		DE	69110060 T	28-09-1995
		DK	574394 T	16-10-1995
		EP	0574394 A	22-12-1993
		ES	2073277 T	01-08-1995
		HU	64569 A	28-01-1994
		JP	6504039 T	12-05-1994
		US	5824640 A	20-10-1998
WO 9602567 A	01-02-1996	IT	1270618 B	07-05-1997
		AU	702294 B	18-02-1999
		AU	3077995 A	16-02-1996
		BR	9508382 A	23-12-1997
		CA	2194861 A	01-02-1996
		CN	1152924 A	25-06-1997
		CZ	9700069 A	13-08-1997
		EP	0770093 A	02-05-1997
		FI	970097 A	06-03-1997
		HU	76328 A	28-08-1997
		JP	10502814 T	17-03-1998
		NO	970114 A	05-03-1997
		TR	960056 A	21-06-1996
		US	5792744 A	11-08-1998
		ZA	9505837 A	21-02-1996
GB 989826 A		NONE		